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The use of systems biology in chemical risk assessment

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Abstract

Risk assessment of toxicological compounds has traditionally relied upon animal experimentation. Omics technologies, especially genomics and proteomics, generate large amounts of data on genome-wide gene expression profiles, protein expression, and protein interaction with xenobiotics (notably toxic ones), enabling the study of chemical action across multiple scales of complexity from molecular to systems levels. This allows detailed exploration of the mechanisms of toxicity. Although all omics technologies may contribute to better understanding of the toxicological impact of chemicals, their application in chemical risk assessment has not yet been recommended for regulatory purposes. With the recent development of the adverse outcome pathway concept, the combination of the modular framework of adverse outcome pathway, together with the network organisation within systems biology, offers an opportunity to shift the paradigm of chemical risk assessment towards a better understanding of chemical toxicology mechanisms. In this review, we discuss the advantages of the use of systems biology tools in chemical risk assessment, as well as the challenges they present, such as model over-parametrisation in quantitative modelling, data gap management in poorly studied substances and the lack of expertise in bridging the new approaches to regulatory levels.

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Keywords

Systems biology, Chemical risk assessment, Systems toxicology, Adverse outcome pathway, Integrated approaches to testing and assessment, Read across.

Introduction

Nowadays, one of the main goals for safety assessment is to replace animal testing with more advanced technologies, which can explain and extrapolate methods both *in vitro* or *in silico* [1]. Traditionally, *in vivo* measurements of lower adverse effect levels and the no observed adverse effect level are used in chemical risk assessment [2]. Furthermore, physiologically based pharmacokinetic (PBPK) modelling is used in risk assessments, where these models serve as adjuncts to modes of action of toxic chemicals. Human health risk assessments have traditionally used dose–response relationships to quantify and characterise the potential adverse health effects in humans. Already in the early 70s, the availability of computers paved the way for the use of PBPK models [3], which since then have been used and approved by the authorities for chemical risk assessment. This has been done through pharmacokinetics, which studies the movement over time of a chemical and its metabolites in biological fluids and tissues [4]. This type of modelling allows the calculation of tissue exposure doses of chemicals and their metabolites over a range of exposure conditions and species [5].

On the other hand, adverse outcome pathways (AOPs) are a relatively recent development, used to describe toxic events. AOPs, first described by Ankley et al., [6] in 2010, were launched by the Organisation for Economic Cooperation and Development (OECD) in 2012 as a pragmatic tool to describe linked causally related events and their result as adverse outcomes. AOPs are formed by a molecular initiating event (MIE) and a series of key events (KEs), organised by increasing complexity, towards an adverse effect [7,8]. Although this tool was developed to be a linear sequence of events, a new approach attempts at branching AOPs to develop AOP networks, which considers the combination of multiple pathways, and linking one MIE with several AOPs [9]. In addition, quantitative AOPs (qAOPs) aim to combine the exposure metrics and toxicokinetics from PBPK models such that they supplement the tools used to support risk assessment [10].

With the increased number of studies integrating the AOP concept, some questions appeared. For example,

the high level of uncertainty from extrapolating between rodents and humans and across other species represents a significant difficulty. qAOP development can require a significant resource investment, and except for rich biological and toxicological knowledge bases on AOPs such as the aromatase inhibition and other related endocrine pathways, it might be not realistic to expect much qAOP development in the near future [11]. Finally, interindividual and intraindividual variability in exposures is growing in importance, but its introduction into risk assessment is very challenging. Also, the homogeneity of the used rodent populations makes it almost impossible to account for human variation [12,13]. Different response thresholds in the different models might also affect dose–response curves [14].

Mechanistic toxicology, the study of how chemicals cause toxicity to living organisms, attempts to unravel the underlying mechanisms and characterise their progression towards adverse outcomes. The description of the relationship between exposure to a toxic substance and the toxicological outcome has not been a major priority in the methods and models used in chemical risk assessment. One of the main challenges in chemical risk assessment remains the high number of chemicals with limited or no toxicological information. Data used for mechanistic understanding in chemical risk assessment rely on a limited number of dose and time points, which makes it difficult to model dose–response curves with low uncertainty. A reductionist approach has governed the chemical risk assessment principles in the past decades [15]. Few specific endpoints were analysed to account for toxicity, which made it more difficult to unravel the complexity of the events underlying a toxic exposure.

Read-across (RAX) approaches, a hypothesis-driven methodology based on data gap filling, has generally been accepted by regulatory agencies, traditionally using chemical similarity between the source and target substance or quantitative structure activity relationships. Such RAX approaches were accompanied by uncertainty factors and weight-of-evidence scores [6,16,17] and suggest an approximation of toxicological events for new and existing toxic chemicals [18]. However, there is no standard method for measuring biological similarity and it has been recently accepted that RAX approach can be strengthened with additional data from a multitude of different approaches, such as *in vitro* screening, omics data, systems biology and their integration through computational models [8]. The use of high-throughput techniques is a step closer to a holistic approach and wider understanding of the mechanisms of toxicity. However, Hartung et al. [19] already outlined the limitations of the approach, pointing out the uncertainty associated with these methods because cell-based assays may not exactly predict relevant biological effects. Besides, single *in vitro* assays are unlikely to yield a perfect result of the toxic event, which highlights the need for integrated testing strategies that can model the toxic event elements to understand each step [20].

Systems biology, comprehensive approaches that help understand the properties of complex, dynamic and nonlinear multilevel biological systems, through the subdomain systems toxicology has the potential to provide the quantitative mechanistic models to address these issues. Systems toxicology can use different levels of information from integrating diverse sources of data as a means to provide deep mechanistic understanding of the underlying toxicological effects, hence allowing for adverse outcome prediction, providing a new paradigm for chemical risk assessment [1].

The behaviour of each factor in a complicated problem in isolation does not explain the overall behaviour of the nonlinear interactions between the biological components. Therefore, systems biology supplements the reductionist approach, by integrating evidence instead of separating the different biological levels — molecules, cells, tissues, organs, individual and population — and aims to better understand the systemic, dynamic state in living organisms [21,22]. It also allows us to investigate the multifunctionality of genes and pathways and help to elucidate the effects of external exposures such as diet, lifestyle and patient care [9].

Data integration and curation in systems biology

The integration of different 'omics' measurements, coming from diverse technologies (genomics, transcriptomics, metabolomics, proteomics, epigenetics), aids to assess chemical effect at the system biology level. The advances in the different 'omics' technologies, as well as the increase in large publicly available data sets, coupled with the development of new modelling approaches have also increased our understanding of toxicological events and effects at multiple biological levels [23]. Among the databases, a short list is presented in the following list:

- TOXsIgN: a cross-species repository for toxicogenomics signatures describing more than 450 distinct chemicals and their 8491 associated signatures [24].
- BD2K-LINCS: 350 data sets have been generated, including transcriptomics, proteomics, epigenomics, cell phenotype and competitive binding profiling assays [25].
- Dixa: a data infrastructure for chemical safety assessment. 95 toxicogenomics studies on 469 compounds [26].
- TOXicology Data NETwork (TOXNET) is a website hub connecting to several toxicology data files [27].
- ToxCast and Tox21contain data on more than 9000 chemicals for more than 1000 different endpoints [28].

- ToxRefBD contains results for thousands of animal toxicity tests [29].
- Chemical Effects in Biological Systems, a database housing data of interest for health scientists, as it displays data in the context of biology and study design. It allows data integration across studies [30].
- ChemProt, a publicly available compilation of chemical–protein–disease annotation resources, of relevance when studying the systems pharmacology of small molecules across multiple layers of complexity, from molecular to clinical levels [31].
- CTD, the comparative toxicogenomics database includes more than 30.5 million toxicogenomic connections relating chemicals/drugs, proteins/genes, diseases, pathways, gene ontology annotations [32].

Systems biology and Integrated Approaches to Testing and Assessment

Systems biology should contribute greatly to the Integrated Approaches to Testing and Assessment (IATA) proposed by the OECD [33]. IATA consists of structured approaches that integrate different types of data and rank them to perform hazard identification, characterisation of drug potency and safety assessment of chemicals [34]. The initial step of IATA is to gather relevant information existing at the specific compound or endpoint levels, from which a first conclusion about chemical risk can be derived. Omics data provide both gene- and pathway-level read-outs (e.g. changes in individual gene expression levels or statistical indices for the differential regulation of sets of genes within a specific biological pathway) that can be used for selecting measurable and relevant biomarkers. For example, Suter et al. [35] have shown that combining transcriptomics with proteomics and metabolomics data improved the ability to provide more specific biomarker candidates for liver hypertrophy, bile duct necrosis and/or cholestasis and proximal tubule damage [35,36]. One of the benefits of such an approach is the use of interconnected data from *in silico* experiments and experimental data (*in vitro, in vivo*).

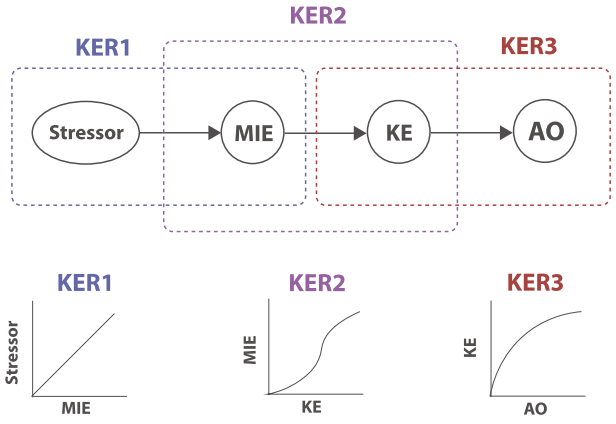
Although IATA provides structured data integration, it does not necessarily offer any mechanistic rationale. On the other hand, AOPs can provide the basis for mechanistic theory (OECD, 2013). AOPs represent chemical agnostic type of pathways, linking a linear sequence of events, from the interaction of substances with initiating molecular events to the generation of key events at the cellular or systems level, which ultimately leads to an observable adverse effect (adverse outcome) at the individual or population level [33]. Thus, the AOP framework describes the progression of a toxicity pathway from the molecular origin to the population outcome, through a series of measurable mechanistic responses that could be integrated with IATA [33,37]. For a more comprehensive and biologically realistic integration of the available knowledge in the form of KEs and key event relationships, Wittwehr et al. [38] described how the use of these aspects can be used as a foundation for construction of AOP networks, transforming the linear sequence of events in AOPs to a network that better explains the biology behind the toxic events.

Systems biology and AOP

Although the AOP and systems biology concepts are different, their complementarity of them might further shift the paradigm of chemical risk assessment towards a better understanding of chemical toxicology mechanisms. AOP describes a transition of system states, integrating different scales of organisation in a linear framework which, as explained previously, can be transformed into a network interconnecting the different modules that form the AOP. Systems biology, having similar qualities as AOP, provides a network organisation which considers the dynamic aspect inside this organisation. Furthermore, mathematical models are often implemented to link the experimental data to biological outcomes in systems biology [39]. For example, dose–time network identification, based on ordinary differential equations, applied on toxicogenomics data, has allowed for deriving mechanisms explaining drug-induced liver injury and carcinogenicity [11].

Contrary to systems biology, the AOP concept has been recognised as a linear and simple model to provide the basis for pragmatic decisions by the regulators. This, together with the limited amount of data and expertise, might explain why the translation of AOPs into

Figure 1

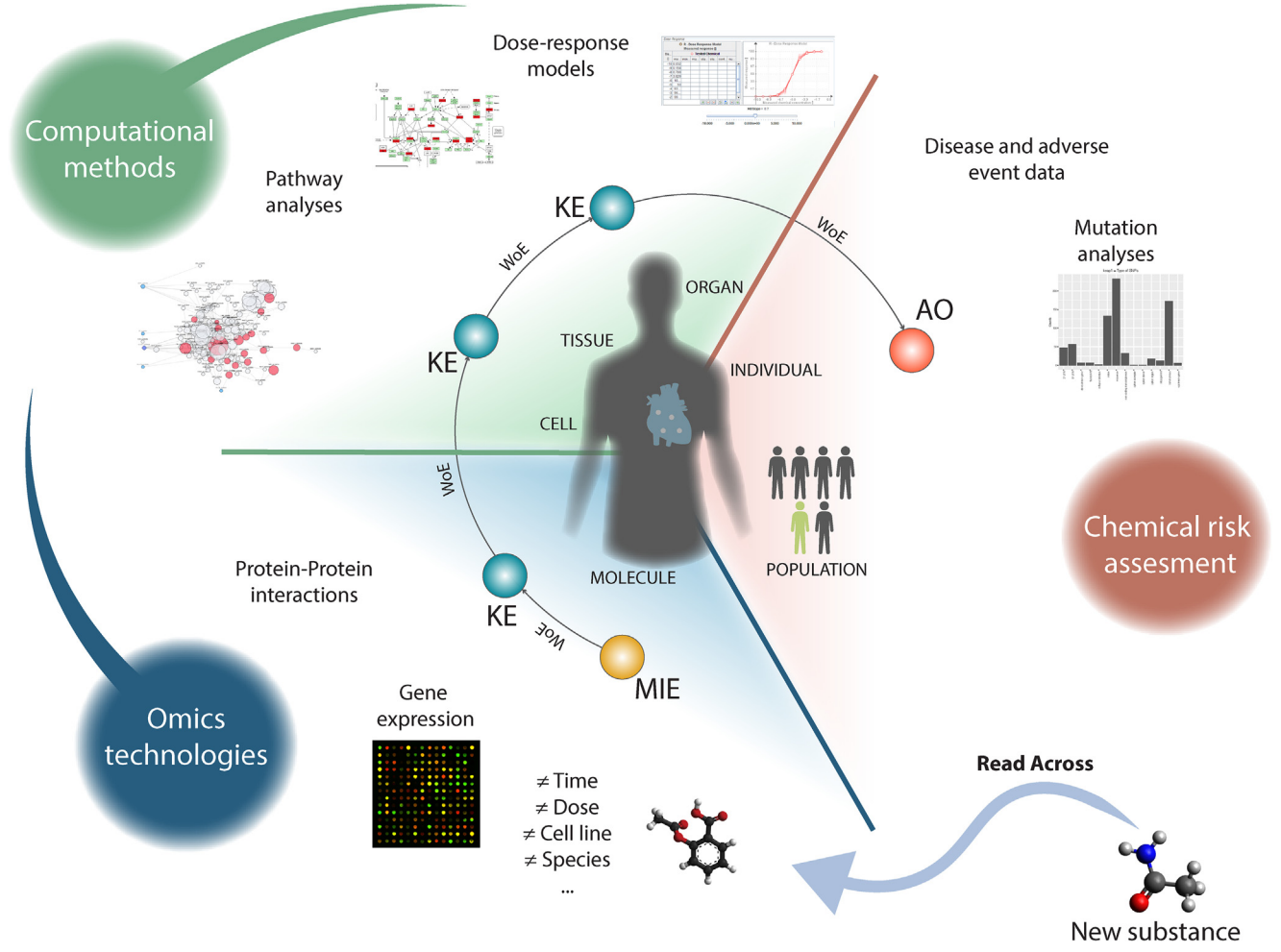


Reductionist approach to model key event relationships. An AOP is formed by a molecular initiating event (MIE) which is triggered by an exposure to a stressor, and it activates a subsequent sequence of key events (KEs) to produce an adverse outcome (AO). By separating each key event relationship, dose–response relationship (stressor-MIE) and activity–response relationship (KE–KE and KE–AO) can be modelled individually. Using this approach, we can understand the individual relationships, which are then reconstructed to the holistic approach to understand the overall quantitative effect of the toxic event. AOP, adverse outcome pathway.

mathematical models remains restricted. However, the AOP networks, as explained previously, help bridge linear AOPs and systems biology by incrementing the complexity of AOPs and developing more biologically relevant frames for them. Promising results start to be reported such as the use of qAOPs that showed population-level decrease in the fathead minnow or its use with synthetic glucocorticoids in fish [40,41]. Some mathematical models applied to AOPs appear feasible, and they may be regarded as transition stages to systems biology models and implemented in the online encyclopedia of AOPs (https://www.effectopedia.org/) [42]. The inclusion of hundreds of independent events is still challenging from the mathematical model point of view, especially because metabolites, genes and proteins play roles in several different pathway at the same time. Among the limitations of mathematical model of complex mechanisms, one is the limited amount of available data that can lead to overfitted models with too many parameters. Using KEs as individual AOP components, it is possible to model the quantitative correlation for each key event relationship (Figure 1).

Thus, genomic data help to identify plausible MIEs. Lan et al. [43] used a toxicogenomics approach to obtain quantitative information for genotoxicity assessment based on DNA damage. Aguayo-Orozco et al. [44] analysed a time-series gene expression data to explore mechanisms of 28 chemical-induced hepatic steatosis toxicities at 3 different concentrations. Protein–protein interaction analysis and other pathway analysis can contribute in deciphering the evolution of the toxicity across all levels of the organism: molecule, cell, tissue, organ and individual (also known as KEs). Repeated analysis at different dosage levels and times can explain the dose–response and time–response relationships.

Figure 2



Integration of systems biology into the AOP concept. Systems biology consists mainly of three parts, starting with the technology used to obtain the different types of omics evidence; computational methods that help understanding the underlying structure and mechanisms within the cell and between molecules and the biological part, in this case chemical risk assessment, giving a biological outcome. All systems biology methodologies, depicted on the outer circle of the figure, describe the different steps that help enrich the knowledge at the AOP level (inner circle). AO, adverse outcome; AOP, adverse outcome pathway; KE, key event; MIE, molecular initiating event; WoE, weight-of-evidence.

With the increasing access to transcriptomic dose–response data in toxicology and risk assessment, genomic dose–response analysis can be performed to support screening-level risk assessments for environmental agents and software, for example, BMDExpress can help for such analysis [43,45].

Although technological advances have facilitated the production of data for mechanistically driven toxicology, we are facing new challenges in how such data are standardised, processed, modelled, interpreted and quantified. Modelling and incorporation of further data, such as disease data, mutation effect studies, patient records and adverse event information, could help to explain the toxic effects at the population level [46]. Ultimately, when a new compound, similar to a previous one, is to be assessed, RAX methods could be exploited to reduce analysis that is expensive and time-consuming. The complementarity of systems biology within the AOP concept is depicted in Figure 2.

There are high expectations of systems biology in chemical risk assessment, although some issues are yet to be tackled:

- Best practices for generating, collecting, storing, curating and integrating 'omics' data are essential to correctly interpret the data. In this context, a database resource (AOP-DB) has been developed, aggregating associations between genes and their related chemicals, diseases, pathways, species, ontologies and gene interactions [47]. Levering the wealth of publicly available data, covering chemical effects on biological systems, can lead to computationally predicted AOPs that might explain the mechanism behind a toxicological event [48].
- Processing of 'omics' data may generate some uncertainties in the interpretation of the outcomes, and some weight-of-evidence approaches should be integrated to make the results originating from these various technologies more reliable. For example, with gene expression data, depending on the technology, the normalisation process, the specificity of the cell types or tissues considered, the dose and time point and the expression change of a gene might be highly significant, resulting in false interpretations. Therefore, the combination of outputs from different technology platforms and data-processing algorithms must be fitted and validated for each study independently.
- The distinction between species needs to be specified in the models. Although at the gene level, the translation from one species to humans is not always clear, and chemical perturbations at the pathway level may be more conserved, facilitating the use of data from different species to extrapolate to humans. Therefore, extrapolation across species can then be improved by focussing on the similarity of biological pathways among species, as opposed to the traditional approach of direct comparison of adverse events, or specific molecules [49–51]. However, it has to be noted that qualitatively similar (or identical) systems may have different quantitative behaviours, and the kinetics are not considered in such extrapolations.

Conclusion

The use of high-content omics data sets has led to numerous publications on chemical MOAs [52–55]. However, it is still a scientific requirement to validate these results with additional biochemical or physiological studies. The AOP approach provides a platform that aims to meet this scientific requirement while supporting the use of omics data sets in risk assessment.

Systems biology through systems toxicology can be applied to develop new models that can assess chemical compounds by RAX, offering a new way of performing chemical risk assessment with better accuracy and lower cost and time consumption. To develop these models, systems toxicology uses existing data on exposure effects of pharmaceutical, industrial and environmental compounds, on different organisms, as well as the existing pathways correlated to adverse outcomes. With the advances in omics technologies and the generation of big data, these models will likely be even more accurate. In addition, the integration of systems biology into AOPs could allow for maintaining the linearity of the biological pathway in AOP and at the same time include the network and dynamic features, contributing to the translation from one observation to another (i.e. from molecular event to cellular event to adverse outcome). This linear depiction of AOPs is convenient and useful, but it is an abstraction of the molecular level biology. The mechanistic biology that underlies AOPs, described in some detail in qAOPs, will extend its use by including feedback and feed forward loops. This could facilitate the transition from the use of omics technologies in research for regulatory purposes.

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Conflict of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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